3-2010

Sucrose, Water and Nutrient Use During State II Multiplication of Two Turmeric Clones (Curcuma longa L.) in Liquid Medium

Jeffrey Adelberg
Clemson University, jadlbrg@clemson.edu

Sucrose, water and nutrient use during stage II multiplication of two turmeric clones (*Curcuma longa* L.) in liquid medium

Jeffrey Adelberg

Department of Horticulture

247 Poole Agricultural Center

Clemson University

Clemson SC 29634

jadlbrg@clemson.edu

864-656-3011 (FAX -4960)

Sucrose, water and nutrient use during stage II multiplication of two turmeric clones (*Curcuma longa* L.) in liquid medium

Abstract

Two clones of turmeric (*Cucruma longa* L.) were assessed nine times during a 28-day culture period in double phosphate MS liquid medium containing 2%, 4% and 6% sucrose. The phase of rapid shoot bud multiplication lasted for 21 days or less, in both clones in all media. The maximum multiplication rate was 3.4x at 20 and 19 days, with 3.9% or 4.7% initial sucrose concentration being optimal for the clones 9-3 and 35-1, respectively. Dry mass increased over the entire culture period and was greatest with 4.8% or at least 6% initial sucrose concentration for the two clones, 9-3 and 35-1, respectively. Maximum tissue water mass required 2.8% and 3.9% initial sucrose, respectively, in the two clones. Tissue relative water content (tissue water mass/ fresh mass) was better correlated with sucrose concentration in medium, than the osmolality of medium, for both clones at all sucrose concentrations. Relative water content increased over time (water was incorporated more rapidly than dry mass in growing tissue), sucrose concentration in media decreased over time (sucrose was used at a relatively more rapid rate than water). The concentrations of 10 nutrients (P, K, S, Zn, Mg, Fe, B, Ca, Mn and Cu) and Na assayed in time course all decreased rapidly over time. The rates of nutrient use were better related to growth of plant mass than shoot bud multiplication. P and Cu were completely depleted from medium before the end of the culture period. P concentration in tissues was in the lower ranges of that considered optimal, and Cu was in excess concentrations, based on comparison to high-yielding field grown plants. Sugar, water, and nutrient use were more closely

related to plant mass than shoot multiplication. The stationary phase of bud multiplication was coincident with continued growth of tissue dry and water mass. Among inorganic nutrients, lowered concentrations of P were most likely related to the stationary phase of bud multiplication, but still permissive of dry and water mass increases. Lowered availability of sugar, other inorganic nutrients, or less available water, did not precede day-21 and the end of active shoot bud multiplication. In batch culture, the concentration of a nutrient that is rapidly used (e.g. sucrose) does not remain at an optimal range for a prolonged period of time.

Index words: batch culture, multiplication ratio, nutrient depletion, osmolality, phosphorus

Introduction

Turmeric is a member of the family, Zingiberarceae, a group of important tropical monocots that includes ginger, cardamom, and the ornamental “gingers”. Turmeric is a sterile triploid propagated by division of subterranean rhizomes. Micropropagation is used for multiplication of disease-free true-to-type nursery stock and facilitates germplasm collections and exchanges in the scientific community (Nimral Babu et. al 2007, and the references contained therein). Clonal micropropagation techniques for turmeric plantlets by repeated multiplication of vegetative axillary buds on semi-solid MS medium were developed using varied concentrations of plant growth regulators, most frequently NAA and BA (Nirmal Babu et. al 2007). Slow-growth is favored by germplasm repositories and combining mannitol and

sucrose in medium allow high survival from plantlets that have not been transferred for several months (Nirmal Babu, et al. 2006).

Turmeric, like other geophytes, may be induced to form in vitro storage organs, by high concentrations of sucrose (6 - 12%) and using liquid medium (Nirmal Babu et. al 2007). Microrhizomes of turmeric are considered an ideal method for producing disease planting material for germplasm exchange. Fed-batch supplementation of medium was used to maintain relatively constant sucrose concentrations that resulted in the rapid formation of microrhizomes (4-weeks) and subsequent formation of secondary "fingers", a morphological feature analogous to the field grown stock plants after15 weeks (Adelberg and Cousins 2007). These well-branched microrhizomes produced vigorously growing nursery stock with 99% acclimatization in small-scale trials involving over two hundred microrhizomes (Cousins and Adelberg, unpublished data). Understanding sugar, water and nutrient use during vegetative multiplication of leafy shoots, biomass accumulation in storage organs, and stationary growth phases would serve scientists and propagators working in the production of clean nursery stock and conservation of elite, sterile germplasm.

This current work examines shoot bud multiplication and biomass accumulation at different initial sucrose concentrations (*Curcuma longa* L). Shoot bud multiplication and growth of biomass was observed by repeated, destructive sampling over the 28-day time course. Two clones were independently assessed under nearly identical treatment conditions to observe responses that would be repeatable across genotypes and specify ranges specific to a chosen genotype. Focus was given to growth during the period of bud multiplication and biomass accumulation that immediately followed. Sucrose use, water availability and nutrient use were correlated during these growth phases.

Materials and Methods

Plant material

Turmeric (*Curcuma longa L.*) accession L35-1 and L93-1 were obtained from the University of Arizona Southwest Center for Natural Products Research and Commercialization. Stage I was prepared by dissecting the quiescent shoot tips from rhizomes, immersing in full-strength commercial bleach (5.25% NaOCl), and plating on hormone free MS media in petri plates. Stage II cultures were maintained for at least 18 months by sub-culture every six weeks in liquid medium, modified MS (Murashige and Skoog, 1962) that included additional, 170 mg NaH_2PO_4, 100.0 mg myo-inositol, 0.25 mg nicotinic acid, 0.25 mg pyridoxine hydrochloride, 0.05 mg thiamine hydrochloride, 60 g sucrose, and 1µm benzyladenine (BA) per liter (Adelberg and Cousins 2006). Medium pH was adjusted to 5.7 before being dispensed. Explants were placed in 180 mL glass jars containing 40 mL of liquid tissue culture medium and cultured on an orbital shaker (100 rpm) with 25 to 35 µmol s^{-1} m^{-2} PAR provided by cool white fluorescent tubes with 16 h d^{-1}photoperiod maintained at 24 $\pm$ 2°C. Fresh cut buds of each clone were pre-conditioned to experimental sucrose concentrations in fresh media for 5-days prior to factorial time course experiment.

Responses in Factorial Time Course

For each clone (L35-1 and L93-1) four buds were placed in each vessel containing 33 mL of medium on shaker, as above. Eighty one vessels, 27 jars of each concentration (2%, 4%, or 6% sucrose) were used for each of the two clones. Nine pre-determined jars of each clone (3 of each concentration) were destructively harvested at each set date (day 1, 3, 7, 10, 14, 17, 21, 24, and

28). At harvest, plants were carefully blotted on paper towels to measure fresh weight (FW), volume of remaining medium was measured in a graduated cylinder, and sugar concentration was determined with a refractometer. Samples of spent medium were frozen in cryogenic storage vials (-20 C) for osmometry and nutrient analysis. Plants were dissected into leaves, rhizomes, and roots, prior to FW and DW determination. Plant dry weight (DW) was recorded after drying tissues from each vessel individually in paper envelopes at 60 °C for 48 h.

Osmometry and Nutrient Analysis

Osmotic potential of spent medium was measured using a Wescor 5520 Vapor Pressure Deficit Osmometer (Wescor Inc., Logan UT). One hundred µL samples were drawn from thawed vials and measured against three point calibrations with clean check, using fresh proprietary osmolality standard solutions supplied by manufacturer. Osmolality of MS medium salts (100%, 50%, 25%, 12.5%, 0%) and sucrose (0%, 1%, 3% and 6%) in complete factorial combinations was determined before and after spent medium samples were analyzed as internal check.

Nutrient analysis was conducted by Clemson University's Agricultural Chemical Service on a Thermo Jarrell Ash Model 61E Inductively Coupled Plasma (ICP). One mL samples of spent medium were drawn from thawed vials and diluted 1:7 in 2x-DI water. Nutrient use, and nutrient concentration in tissue dry matter were calculated considering the vessel as a closed system with respect to inorganic ions, and nutrient mass not present in the spent medium was incorporated in plant mass (Adelberg et al. in press).

Experimental Design and Analysis

On each of nine harvest dates, for three sucrose level, and two clones, three vessels were assigned in a pre-determined completely randomized design (9 x 3 x 2 x 3 vessels). The main and interactive effects of days in culture, initial sucrose concentration and clone, in full factorial arrangement were partitioned by ANOVA, prob. $F < 0.05$, for each of the response variables. Best linear and polynomial model fits were presented in line graphs. Maxima were determined using response surface methodology with second order interactions and quadratic responses fit for day x sucrose, with both clones (JMP 8.0, SAS Inst., Cary, NC, USA).

Results and Discussion

Shoot bud multiplication was rapid during the first few days of time course experiment. In the lowest sucrose treatment (2%), bud multiplication apparently had ceased after 15 days, when the multiplication ratio about 2.5x (Fig. 1a and 2a). For both clones at all sucrose concentrations, bud division ended after the first 21-days of culture. Bud count data often lacks a normal distribution and than the simple process of comparing multiplication ratios by ANOVA, would not be valid, and Poisson regression of logistic relationships is generally preferred (Mendes et al. 1999). The bud count data in this current work produced near normal distributions for both clones and ANOVA was used as an appropriate analysis.

There was an apparent 4-day lag phase in clone 9-3 for in the 6% treatment, before bud multiplication became apparent. In the 4% sucrose treatment, multiplication continued until day 19, and was about 3x. In 6% sucrose treatment, the maximum multiplication ratio was reached at about 21 days. It should be noted that all buds in this experiment had been cut 5 days prior to being placed in the time course observations, and a lag phase of less than 5 days may have gone

unobserved in the other treatments. Bud number appeared to decrease as the plantlets grew larger and buds may have been hidden in leaf, root and rhizome epidermal tissues (Fig. 3) resulting in an apparent decrease in multiplication ratio. When observing stage II multiplication of seas oats, Valero-Arcama et. al (2008) also recorded an apparent decrease in shoot numbers as days in culture proceeded past an optimal date. Bud multiplication of turmeric ceased after 21 days in both clones and at all concentration of sucrose and may be viewed as the end of the exponential growth phase (with regards to multiplication of buds). The maximum multiplication ratio was 3.4x for both clones after 20-days and 19 days (clone 9-3 and 35-1, respectively) with 3.9% and 4.7% initial sucrose, respectively (Electronic Supplemental Figure A).

Turmeric remains in an active growth phase in terms of plant mass, after it has entered a stationary phase regarding bud division. Dry mass increased over the entire 28-day culture cycle for both clones at all sugar concentrations. The lowest sugar concentration had the least dry mass gain (Fig 1b and 2b). This is consistent with observations of dry mass in turmeric being related to sucrose uptake from the medium (Cousins and Adelberg 2008). In clone 9-3, the 4% treatment had more rapid rate of dry mass increase than the 6% treatment and the optimal initial sucrose concentration for dry mass was 4.8% sucrose. In clone 35-1, the rate of dry mass gain continually increased and the 6% treatment had greater mass than the 4% treatment by the end of the culture period. Six percent sucrose concentration (or greater) was optimal for dry matter accumulation in clone 35-1.

Water mass is the largest component of fresh biomass. Tissue water uptake, the difference between fresh and dry mass, responds differently to sucrose than dry mass. Early in the culture cycle with both clones, water uptake was most rapid in the 2% sucrose media, intermediate in the 4%, and slowest in the 6% sucrose medium (Fig. 1c and 2c). In clone 35-1 in

175 the 2% sucrose medium, plantlets stopped incorporating water by day 24, but the 6% sucrose
176 medium had increased water uptake over the entire 28 days, and so tissue water mass was similar
177 for the high and low sucrose concentrations by the end of the culture cycle. In both clones in the
178 4% sucrose medium, the rate of water uptake increased over the entire time-course and the
179 optimal initial sucrose concentration for maximum water uptake was 2.8% and 3.9% for clones
180 9-3 and 35-1, respectively.

181 Sucrose concentrations in all media declined over time (Fig 1c and 2c) and therefore
182 sucrose use was relatively more rapid than water uptake. Sucrose concentration decreased most
183 rapidly in the latter stages of growth in the 4% sucrose medium when water uptake was most
184 rapid. Sucrose concentrations between 0.5 and 2% toward the end of the 28-day period were
185 observed in 2% and 4% treatments and favor the uptake of water, but not dry mass accumulation.
186 Sucrose was still present in medium when clone 35-1 entered stationary phase of water uptake
187 and the depletion of sucrose did not coincide with the stationary phase. There was also an ample
188 volume of water present in all of the vessels over the entire time course (data not presented).
189 Some other factor would be limiting water uptake in clone 35-1 in the low sucrose medium.

190 In both clones, plants in the 2% sucrose medium had a high relative water content
191 (>92%) during the entire 28-days. With 4% sucrose concentrations, the relative water content of
192 tissue increased over the entire culture cycle was approximately the same as the 2% medium by
193 day 28 (Fig. 1d and 2d). Water was incorporated into plant mass at a relatively more rapid rate
194 than media solutes. The relative water content in 6% sucrose medium also increased over the
195 entire culture cycle by day 28 approached 92%. There was an inverse correlation between
196 sucrose concentration in medium and the relative water content in tissue for both clones, at all
197 sucrose concentrations (Fig. 4) similar to many different plants (Adelberg 2006; George et al.

198 2008; and references contained therein). As sucrose declined over time, the relative water
199 content of the plants increased. In both clones, relative water content did not increase past
200 approximately 95% (Fig. 1e and 2e).
201 Sucrose mass is several time greater than all other media solutes combined, and is the
202 largest component of dry mass accumulation. Besides its nutritive role in building plant mass,
203 sucrose concentration affects the medium osmotic potential. For example MS medium with 3%
204 sucrose has roughly an equal osmotic contribution from inorganic salts and sucrose. Sucrose and
205 nutrient salt uptake both effect the osmolality of spent MS medium (Supplemental Fig. B).
206 Osmolality, the combined number of solute particles without regard for size, density or charge,
207 quantifies the medium's osmotic effect on tissue water potential (George et. al 2008). Osmolality
208 decreased over the time course, for all three sucrose treatments in both clones (data not shown).
209 In the 2% sucrose medium, by the end of the culture period, osmolaltiy was reduced from 175
210 mOsm/kg to 50 mOsm/kg, water was more available and osmolality did not limit water uptake.
211 Relative water content in tissue had a poor correlation with osmolality for both clones at all of
212 the sucrose levels (Fig. 5). An apparent paradox exists, where sucrose concentration was better
213 related to the relative tissue water content than the combined colligative effects of all solutes.
214 The active uptake of nutrient ions against concentration gradients would partially explain the
215 paradox.
216 Decrease in specific nutrient concentrations in medium was analyzed for 11 ions (P, K, S,
217 Zn, Mg, Fe, Na, B, Ca, Mn, and Cu) during the time course. The decrease was well correlated
218 with plant dry mass (Electronic Supplemental Figure C) and some general statements about
219 nutrient use were valid. At termination of the culture period, measurable quantities of nutrients
220 were present except for P and Cu (Fig. 1f, 2f and data not presented). S and Zn were predicted to

approach depletion near the end of the culture cycle, based on short-term extrapolation (data not shown). For all of the nutrients (except Cu) media with highest sucrose concentrations had slowest rates of nutrient use. Phosphorus was unique among nutrients in being completely expended during the time-course, with the greatest sugar concentrations being depleted last. Shoot bud division was slowing while phosphorus became scant. Phosphorus has been observed to limit growth in MS formulations (George et al. 2008) and this current research was conducted with 2.48 mM double- phosphate formulation of Murashige et al. (1972) for asparagus shoot cultures. When phosphorus in tissue dry matter was estimated, P was found to be in the lower portion of the optimal range that defined the foliar diagnostic norm for field grown turmeric (Kumar et al. 2003).

Copper was depleted very rapidly from the medium. However, copper was always in excess of optimal concentrations in tissue dry matter as compared to field-grown tissue (Kumar et al. 2003). Plants may continue to grow for a short period after depletion of nutrients from medium and this defines luxuriant consumption. Luxuriant consumption of P, Cu, S and Zn would than allow the increase in mass to continue past the time of nutrient depletion.

Nitrate and ammonium were not analyzed in this current work. Increased biomass (fresh and dry) and shoot bud multiplication ratios resulted from reducing MS ammonium, nitrate and potassium to 25% (in equi-molar ratios) of that specified in MS formulation, with liquid culture of turmeric clone 35-1 (Adelberg and Halloran, unpublished data). The ratio of N and K may be out of balance with regards to P, but more complex multifactor designs are required for this determination.

Discussion

Stage II micropropagation induces axillary shoot proliferation and the growth of shoots to sufficient size to become new stage II explants, or pass to stage III (George et al. 2008). An obvious objective of efficient micropropagation is the increase in numbers of new shoots. Soot bud development stopped by day 21, while the cultures were still actively growing and ample sugar and water, were available in medium. Quantifying the multiplication ratio of a stage II operation must factor in the multiplication ratio of a single cycle, the length of time required for that cycle, and then subtract for product loss (Hara and Kozai 1992). Rapid micropropagation of turmeric would best be accomplished by "short-cutting" the subculture cycle to less 21-days or less. This would allow the most plants to be produced in a calendar year, by maximizing the number of culture cycles that could be accomplished without reducing the number of plants produced. This would work well for both clones. An unintended secondary benefit of "short-cutting" would be fewer roots and foliar materials to be removed, and so the hood worker would require less time in the transfer process. Repetitive stage II hood multiplication is the single most expensive component of any tissue culture operation.

Plant micropropagation in small, sealed vessels may be considered as a batch process with three phases of growth: induction of cell division, exponential growth, and a stationary phase. Subculture eventually becomes necessary because of the depletion of an essential nutrient(s), drying of media, or the build-up of toxic substances (George et. al 2008). To the knowledge of this author, the phases of a subculture cycle have not been well characterized for micropropagation.

Nutrient depletion did not precede the cessation of shoot multiplication, however phosphorous was disappearing while the increase in bud number was slowing. Bud multiplication starts immediately after subculture to fresh media, when the physical cutting of the plant into single bud units eliminates apical dominance. Nutrients, like phosphorus, may be necessary in greater concentrations, or periodic infusion of phosphorous may help to maintain bud multiplication over a longer duration of the culture cycle. An alternative, non-nutritive speculation for the cessation of shoot multiplication may be apical dominance imposed by the enlarged shoot system. Media supplementation might not extend the length of the multiplication cycle or alter the requirement for frequent cutting. The lack of nutrient, sugar, or water availability did not cause the cessation of biomass growth.

Greater mass makes better plants for acclimatization and microrhizomes have been very convenient for moving turmeric plantlets to ex vitro conditions. In vitro rhizome mass is directly related to sugar-use and short-term batch culture models (5-weeks) have predictive value for long term cultures (210 weeks) in fed-batch bioreactor vessels (Cousins and Adelberg 2008). Our current 28-day time course experiment showed mass increase would continue over time, if medium concentrations maintained adequate sucrose concentrations. Sucrose was used most rapidly in the 4% medium with the decrease over time becoming most rapid toward the end of the time course. Sucrose would become deplete if plantlets were to remain in the 4% medium for periods longer than the 28-days in these trials. Increasing the sucrose concentration to 6% slows the uptake of water. Therefore periodic supplementation of medium may be necessary to maintain optimal rates of mass increase. Using fresh medium supplements creates the likelihood that nutrients in the least demand, build up at the greatest concentrations, as large amount of water are used by large plant mass. Na, Ca, Fe and Mn were the minerals least used in the 28-

day culture and may not be needed in fed batch supplements. P, and then S, Mg, K, and Cu may be the minerals most needed in supplements for long-term culture of storage organ biomass.

Conclusion

We are frequently are asked to develop medium to maximize a response. This experiment showed that optimizing a medium for a single component like sucrose and was dependent on the genotype and the type of growth desired (buds, fresh or dry mass). Maximizing the multiplication phase involves cutting the plants at day 20 or 19, for the clones 9-3 and 35-1. In common practice a single consensus medium and 3-week culture period would selected for optimal plant multiplication. Larger sized plants were obtained by leaving the cultures for a longer period (and four weeks was not enough to obtain full dry mass). The optimal initial sucrose concentration for clone 9-3 was 3.9% for multiplication, 4.8% for dry mass and 2.8% for water mass. The optimal initial sucrose concentration for 35-1 was 4.7% for multiplication, 3.9% for water mass, and would need to be at least 6% for dry mass. In 28-days, sucrose concentrations of both clones decreased sharply. Over the 2%- 6% initial range tested, increasing sucrose concentration prolonged the period of dry matter accumulation. The decreased initial sucrose concentration shortened the duration of bud division and dry matter accumulation. The rapid decrease in sucrose concentration during the 28-day cycle at “near optimal” sucrose concentration (e.g. 4%) showed that maintaining dry matter accumulation over a longer time course would require supplementation of the batch culture.

Dry mass growth and bud multiplication required greater nutrient concentrations in media than uptake of water in fresh mass. Bud multiplication ceased while dry mass continued to

increase and so bud multiplication may require still higher nutrient concentrations. Certain nutrients like P and Cu were used most rapidly. Developing a longer duration of multiplication cycle, during micro-rhizome propagation or conventional leafy shoot propagation, requires fed-batch supplements of nutrients that complement what has been depleted from the medium. Liquid-cultures allow experimentation and optimization to approach the concept of "replacement" for nutrients expended in bud division and rhizome growth.

Acknowledgements

The author thanks Anne O'dell and Stephanie Whitson, who conducted much of the wet laboratory work, the summer they were admitted to Clemson University, as part of the Eureka Program in the Calhoun Honors College that introduces undergraduates to research.

References

Adelberg, J., Delgado, M., Tomkins, J. 2009. Spent medium analysis for liquid culture micropropagation of *Hemerocallis* on Murashige and Skoog medium. In Vitro Cellular and Dev. Biol.- Plant DOI 10.1007/s11627-009-9247-1.

Adelberg, J. and M. Cousins. 2007. Development of Micro and Minirhizomes of Turmeric, *Curcuma longa* L., in vitro. Acta Horticularae 756, 103-108.

Adelberg, J. 2006. Agitated thin films of liquid media for efficient micorpropagation. In: Dutta-Gupta, S., Ibaraki, Y. (Eds.) Engineering for plant tissue culture Vol. 6., Springer-Verlag, Heidleberg, Germany, 101-117.

335 Cousins, M., Adelberg, J. 2008. Short term and long term time course studies of turmeric
336 (*Curcuma longa* L.) microrhizome development in vitro. Plant Cell Tissue and Organ
337 Culture 93,283-293
338 Hara, M., Kozai, T. 1992. Mathematical methods to maximize the overall multiplication
339 ratio of micropropagation in a determined period. Acta Hortic. 319,625-630.
340 George, E.F., Hall, M.A., De Klerk, G.J. 2008. Plant Propagation by Tissue Culture,
341 Ed.3, Vol. 1. Springer, Dordrecht, Netherlands.
342 Kumar, P.S.S., Geetha, S.A., Savithri, P., Jagadeeswaran, R., Mahendran, P.P. 2003.
343 Diagnosis of nutrient imbalances and derivation of new RPZI (Reference Population
344 Zero Index) values using DRIS / MDRIS and CND approaches in leaves of turmeric
345 (*Curcuma longa* var.) J. Appl. Hort. 5,7-10
346 Nirmal Babu, K.N., D. Minoo, S.P. Geetha, V. Sumanthi, K. Praveen. 2007.
347 Biotechnology of turmeric and related species. *In* P.N. Ravindran, KN Babu, K
348 Sivaraman, eds. *Turmeric: The genus Curcuma*. CRC Press, Boca Raton, 107-127.
349 Mendes, B.M..J., Filippi, S.B., Demetrio, C.G.B., Rodriguez, A.P.M. 1999. A statistical
350 approach to study dynamics of micropropagation rates, using banana (*Musa* spp.) as
351 an example Plant Cell Reports 18,967-971
352 Murashige, T., Shabde, M.N., Hasegawa, P.M., Takatori, F.H., Jones, J.B. 1972.
353 Propagation of asparagus through shoot apex culture. I. Nutrient medium for the
354 formation of plantlets. J. Amer. Hortic. Sci. 97,158-161.
355 Murashige, T., Skoog, F. 1962. A revised medium for rapid growth and bio-assays with tobacco
356 tissue cultures. Physiol. Plant. 15,473-497

Valero-Aracama, C., Kane, M.E., Wilson, S.B., Philman, N.L. 2008. Comparative growth morphology and anatomy of easy- and difficult-to-acclimatize sea oats (*Uniola panniculata*) genotypes during in vitro culture and ex vitro acclimatization. J. Amer. Hort. Sci. 133,830-843

Walker, P.N. 1995. Systems analysis and engineering. In: Aitken-Christie, J., Kozai, T., Smith, M.A.L. (Eds.) Automation and environmental control in plant tissue culture. Kluwer Academic Publishers, Dordecht, Netherlands, 65-86.

Figure captions

Figure 1. Turmeric clone 9-3 with 2% (···△···), 4% (–○–) and 6% (---□ ---) sucrose in a double phosphate MS medium was routinely sampled during a 28-day culture cycle. Multiplication ratio (A), tissue dry mass (B), tissue water mass (C), sucrose concentration in spent medium (D), relative water content of tissue (E), and the % of phosphorus used during the culture cycle, are shown with respect to days in culture. Best linear and polynomial model fits as determined by ANOVA, prob. F <0.05, for each of the response variables were presented.

Figure 2. Turmeric clone 35-1 with 2% (···△···), 4% (–○–) and 6% (---□ ---) sucrose in a double phosphate MS medium was routinely sampled during a 28-day culture cycle. Multiplication ratio (A), tissue dry mass (B), tissue water mass (C), sucrose concentration in spent medium (D), relative water content of tissue (E), and the % of phosphorus used during the culture cycle, are shown with respect to days in culture. Best linear and polynomial model fits as determined by ANOVA, prob. F <0.05, for each of the response variables were presented.

Figure 3. Turmeric clone 9-3 was shown after 13 days of liquid culture with 6% sucrose has highly visible bud divisions (A). After 28-days (B), bud divisions become obscured by the growth of foliage and roots, in 2%, 4% and 6% sucrose concentrations (left to right, respectively).

Figure 4. Relative water content of two turmeric clones (9-3 and 35-1) in 2% (△), 4% (○) and 6% (□) sucrose in double phosphate MS medium was related to sucrose concentration in spent medium as sample routinely during a 28-day culture cycle. Best linear model fit as determined by ANOVA, prob. F <0.05, with shaded area showing line with 95% confidence.

Figure 5. Relative water content of two turmeric clones (9-3 and 35-1) in 2% (△), 4% (○) and 6% (□) sucrose in double phosphate MS medium was related to osmolaltiy of spent medium as sample routinely during a 28-day culture cycle. Best polynomial model fit as determined by ANOVA, prob. F <0.05, with shaded area showing line with 95% confidence.

Electronic Supplemental Figure A. Multiplication ratio of turmeric clone 35-1 with 2%, 4% and 6% sucrose in a double phosphate MS medium was routinely sampled during a 28-day culture cycle. Response surface methodology was used to predict best fit model of “days x sucrose” for linear, quadratic and interactive terms with 95% confidence.

Electronic Supplemental Figure B. Dilutions of MS salts, at varied sucrose concentrations, affect the osmolality and were used as checks for the sampled media. Distortion of the linear contours below 20% MS shows the range of the instruments sensitivity.

Electronic Supplemental Figure C. Scatterplot-matrix shows relationships between dry weight, bud count, and concentrations of 11 ions in spent medium sampled routinely during 28-day time course experiment with two turmeric clones (9-3 and 35-1) in 2% (△), 4% (○) and 6% (□) sucrose in double phosphate MS medium. Ellipses bound 95% of the populations and response plots approaching linearity are more directly correlated.